Optical Trapping and Manipulation of a Single Human Virus

Presented by:

OSA Optical Trapping and Manipulation in Molecular and Cellular Biology Technical Group
Optical Trapping and Manipulation in Molecular and Cellular Biology (BT)

Optical trapping has been widely used to uncover fundamental aspects of molecular and cellular biology, including the understanding of the movement mechanisms of molecular motors and the forces involved in cell adhesion. This group focuses on the development and application of novel optical trapping and manipulation techniques to biological problems. Focus areas include the use of evanescent fields and state of the art optical tweezers for molecular- and cellular-scale manipulation, integration of optical manipulation with microfluidics and lab-on-a-chip technologies, as well as optical sorting and optical methods for cell biology.

GROUP LEADERSHIP

<table>
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<tr>
<th>Name</th>
<th>Affiliation</th>
<th>Title</th>
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<tr>
<td>Steven Leonard Neale</td>
<td>University of Glasgow</td>
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<td>Peter Pauzauskie</td>
<td>University of Washington</td>
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<td>Peter John Reece</td>
<td>University of New South Wales</td>
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<td>Stephanie Jones</td>
<td>University of Victoria</td>
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<tr>
<td>Daniel Richard Burnham</td>
<td>University of Washington</td>
<td>Secretary</td>
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UPCOMING MEETINGS

RECENTLY PUBLISHED

Announcements

Join the Optical Trapping and Manipulation in Molecular and Cellular Biology Technical Group for their inaugural webinar on Thursday, 16 June 2016, at 11:00 EDT.

Dr. Wei Cheng from the University of Michigan will present his work demonstrating that a single HIV-1 virion can be stabilized, trapped, manipulated, and measured in physiological media with high precision.

Register for the Webinar Now>>

View the feature issue of Biomedical Optics Express on Optical Trapping Applications online now. The issue presents studies that were the focus of the OTA Topical Meeting, which was held in April 2015 in Vancouver, Canada.

Join our Online Community

Stay connected with the Optical Trapping and Manipulation in Molecular and Cellular Biology Technical Group by following #BTTechGroupOSA on Twitter.

#BTTechGroupOSA

www.osa.org/BT
Twitter - #BTTechGroupOSA

Announce new activities

Promote interactions

Contact your Technical Group and Get Involved!
Webinars (today is our first!)
Previous webinars will be available for viewing at the OSA Technical Group website

Panel discussions, discussion forums, and social gatherings at conferences
Look for us at the Optical Trapping Applications (OTA) and other conferences

Facebook page
Optical Cooling and Trapping (OT) Technical Group;
https://www.facebook.com/groups/187451984746395/
Welcome to Today’s webinar!

Dr Wei Cheng – University of Michigan

OPTICAL TRAPPING AND MANIPULATION OF A SINGLE HUMAN VIRUS WEBINAR

16 June 2016 • 11:00 EDT

Register today for this free OSA Technical Group webinar
Optical Trapping and Manipulation of a Single Human Virus

Wei Cheng
Associate Professor
Pharmaceutical Sciences
Biophysics
Biological Chemistry
University of Michigan, Ann Arbor
Trapping of Single Tobacco Mosaic Virus

Optical Trapping and Manipulation of Viruses and Bacteria


TMV, 300 nm long, 18 nm diameter
Challenges for Trapping Animal Viruses

(1) Small size
small dipole moment

\[ \mathbf{P} \propto d^3 \]

TMV has permanent dipole Moment,
Biopolymers (1976) 15: 301

(2) Index of refraction unknown

glass bead

1 \, \mu m
Technical Elements to Prepare for Trapping of a Single Virus

(1) The choice of trapping laser wavelength: 830 nm instead of 1064 nm
Less heating, free of oxygen-mediated photo damage

(2) Back-focal-plane interferometry with high accuracy:
Diffusion coefficient, corner frequency, particle diameter, trap stiffness

(3) Simultaneous two-photon fluorescence excitation by the 830 nm trapping laser
with single-fluorophore sensitivity

830 nm CW laser can excite GFP

Optical Trapping of HIV-1 Virions in Culture Media

EGFP labeled HIV-1
Kim...Cheng, PLOS One (2013) 8: e67170.

10 µm

(in complete culture media: 90% DMEM + 10% FBS)
Polydispersity of HIV-1 Virions

\[ \Phi_{\text{ave}} = 148 \pm 24 \text{ nm} \]

\[ \Phi_{\text{ave}} = 145 \pm 25 \text{ nm} \]


Optical Trap Stiffness and Materials

Bustamante, Cheng & Mejia, *Cell* (2011) 144: 480
Relate Optical Trap Stiffness to Particle Size

Rayleigh particle, stiffness $\propto R^3$

$4\pi n_0 R/\lambda \ll 1$, in reality $> 1$
An Analytic Solution to Optical Trap Stiffness

\[ \kappa = \alpha I_0 \omega_0 \frac{2\pi}{\xi^3} \left[ \sqrt{2\pi} \left( (\xi a)^2 + \frac{1}{4} \right) e^{-2a^2} \text{erfi}(\sqrt{2a \xi}) - \xi a e^{-2a^2} / \epsilon^2 \right] \]

Applies to spherical particles < 160 nm radius

Stiffness = \( F(\text{particle radius, particle refractive index, beam parameters}) \)

Measure Refractive Index for Single HIV-1 with High Precision

Stiffness = $F(\text{particle radius}, \text{particle refractive index}, \text{beam parameters})$

Mean ± SD  
1.42 ± 0.02

CV  
1.4%

74 ± 12 (nm)

16%

Compared to 1.58 of polystyrene; close to 2M sucrose; first time that the RI of a single virus was ever measured.

Parameters from Optical Trap

- Diffusion coefficient
- Corner frequency
- Particle diameter
- Trap stiffness
- Index of refraction


- Two-photon fluorescence with single-molecule sensitivity


Multi-parameter analysis and potential sorting of biological nanoparticles

Current flow cytometry >300 nm

DeSantis & Cheng, *WIREs Nanomedicine and Nanobiotechnology* (2016)
Two-photon Fluorescence with Single-Molecule Sensitivity

IR laser; >100 mW

160 nm

410 nm

Excitation volume = 44 aL
1 molecule / 44 aL: 38 nM

Heterogeneity Matters for Viruses

Model of HIV-1 virion

Presumed gp120/gp41

Gp120/gp41

Cell

Optical Trapping ‘Virometry’

DeSantis...Cheng, *J. Biol. Chem* (2016) 291: 13088
Cooperativity among Gp120 Molecules

Prepared seven populations of HIV-1, each population with on average different density of gp120 molecules.

Virometry to measure # of gp120
Cell culture assay for infectivity

\[
\text{Infectivity}^\% = \frac{s^h}{T_{1/2}^h + s^h}
\]

Clinical Implications

• Are they prone to transmission?
• Transmitted virus are enriched for higher gp120 content.

Not every HIV-1 virion is created equally!

Parrish et al. PNAS (2013) 110: 6626
Single-Cell Manipulation for Single Virion Delivery

Hou & Cheng, unpublished

Suction force controlled by hydrostatic pressure

Scale bar: 10 µm
Specific Association upon a Single Collision is Rare

“Forced” delivery of HIV to CD4+ T cells

- An optically trapped virion is slowly brought into contact with a cell
- Attachment is easy to detect as an immobilized virion remains in focus
- The laser is turned off once the virion escapes

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<th>Trials</th>
<th>Attachments</th>
<th>Attachment Probability (%)</th>
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<tr>
<td>73</td>
<td>0</td>
<td>0</td>
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GFP labeled HIV-1, SUP-T1 cell, 20°C PBS

DeSantis & Cheng, unpublished
Frequent but Nonspecific Association Promoted by DEAE-dextran

(10 μg/mL DEAE-dextran)

Polycation ‘bridge’


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<tr>
<td>77</td>
<td>59</td>
<td>&gt;77</td>
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GFP labeled HIV-1, SUP-T1 cell, 20°C PBS

*DeSantis & Cheng, unpublished*
Possible Reasons for Lack of Attachment and Potential Future Development

- The virion may have low gp120;
- Cell surface receptor may be low or distribution of receptors is heterogeneous

To form specific contact upon a single collision is a rare event!!
Conclusions

- For the first time, optical trapping of a single human virus
- For the first time, measurement of the index of refraction for a single virus particle
- Optical trapping virometry for multi-parameter analysis of biological particles.
- HIV-1 gp120 displays a positive cooperativity in mediating HIV-1 infection.
- The technique to deliver a single virus to a single cell.
Acknowledgments

Past lab members:
Yuanjie Pang, Ximiao Hou
Jin H. Kim, Hanna Song

Current lab members:
Mike DeSantis, Abhay Kotnala
Chunjuan Tian
Zhilin Chen, Tai-wei Li
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Postdoc wanted!